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Glutathione (GSH)-Induced Bud Initiation in *Hydra oligactis*

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Budding in the genus Hydra *was first described in the scientific literature, and sketched, by Leeuwenhoek (1). Since that time, budding has continued to be a popular problem for developmental biologists who study* Hydra *(2, 3, 4). The relationship between feeding and budding was established in mass culture experiments performed by a number of investigators (5, 6, 7, 8, 9). The discovery by Schaller (10) of a low-molecular-weight peptide released by the nerve cells of* Hydra *in response to feeding or injury, and her determination that this polypeptide acts as a mitogen (11), taken together with studies showing a correlation between the mitotic index and the budding rate in* Hydra *(7, 12, 13) left little doubt that feeding and budding are causally related. Recent studies continue to reinforce the observation that reduced glutathione (GSH), or glutathione derivatives, can elicit the feeding response in* Hydra *(14, 15). But the literature is mute about the possibility that GSH, which causes a mechanical feeding response in* Hydra *(16), might also stimulate budding. This study was designed to test that possibility. The specific objectives were (a) to determine whether exposure to GSH leads to a significant increase in the rate of bud initiation in starved* Hydra oligactis; *(b) to determine by employing decapitated* H. oligactis, *whether any observable GSH effect can occur without mediation by the head of the hydra; and (c) to determine whether any GSH induction of budding is additive to that elicited by feeding. All of these objectives were met, and we propose that GSH plays a (significant) role in the cascade of events heading to budding in* Hydra.

H. oligactis individuals were purchased from Carolina Biological Supply Co. (Burlington, North Carolina) and subcultured in an artificial pondwater (APW) medium (17) at 19°C. The animals were fed *Artemia salina* (Wards) nauplii daily, and the medium was changed approximately 3 h after feeding. Mass culture were kept in 8″ Pyrex dishes. All hydras used in this study possessed one stage-one bud (9). All animals were starved for 24 h before use.

A 10^{-5} molar solution of GSH was prepared with APW. Where GSH treatment is indicated, the culture medium contained 10^{-5} *M* GSH. The medium was discarded each day and replaced with fresh medium containing GSH. For the groups exposed to APW alone, the medium was also changed daily. Seventy-five animals were used for each treatment. Each animal was kept in an individual, 16 × 50 mm, numbered Petri dish to allow for individual data collection.

Following initial 24-h pre-experimentation starvation periods, hydras were treated in the following ways.

(a) Unfed controls were starved throughout the 96-h period of the study.
(b) Animals fed on alternate days were fed on days one and three of the study.
(c) Alternate day-fed animals with GSH exposure were treated as those in (b) above but with the addition of 10^{-5} *M* GSH to the medium.
(d) Hyposomally transected animals (*i.e.*, "decapitated") were prepared at the initiation of the GSH observation period and were continuously starved for the entire 96-h period.
(e) Finally, decapitated and GSH-exposed animals were treated as in (d) above, except that 10^{-5} *M* GSH was added to the culture medium.

Recorded observations of bud initiation reflect new buds only. The original stage-one of each animal was not

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Table I

The effect of glutathione (GSH) on bud initiation in Hydra *under various treatments*

Treatment	Incubation time			
	24 h	48 h	72 h	96 h
Unfed control	2	9	14	22
Unfed with GSH	22*	41*	58*	66*
Alternate day fed control	3	19	29*	43*
Alternate day fed with GSH	15*	56*	65*	83*
Decapitated control	3	29	35	38
Decapitated with GSH	22*	56*	63*	82*

Brackets indicate pairwise comparisons.
* $P < 0.05$, *t*-test.
n = 75 for each treatment group.

included. Point-to-point comparisons of bud initiation were performed with a two-tailed *t*-test. Bud numbers were assessed at 24-h intervals.

All of the data are presented in Table I. When starved *H. oligactis* bearing a single stage one bud are exposed to reduced glutathione, the rate of bud initiation is significantly higher than that of unexposed controls during all three observation intervals. By the end of the 96-h incubation period, the GSH-treated animals displayed a 3-fold increase in the rate of bud initiation. Moreover, decapitation had no influence on this GSH-induced budding (Table I): at all three observation intervals, decapitated animals treated with GSH displayed significantly elevated rates of bud initiation when compared with headless but untreated controls.

When hydras were fed on alternate days, an increased rate of bud initiation was expected. Table I also shows that animals fed on alternate days have a higher rate of bud initiation than starved controls. Although alternate day feeding significantly increased the rate of bud initiation, the addition of GSH led to a further significant increase over the already accelerated rate caused by feeding alone (Table I). We selected alternate day feeding as an experimental condition because we had already determined that daily feeding produces a maximum budding rate, thus obscuring any additional effect of GSH (unpub.).

In summary, our results clearly show that at all recorded intervals and under all experimental conditions, hydras treated with GSH have significantly higher rates of bud initiation than untreated control animals. Further, because GSH was fully effective when tested on decapitated animals, the mechanical feeding response cannot be responsible for the increased bud initiation. We have also shown that the effect of GSH is additive to that elicited by feeding and decapitation; *i.e.*, the combined effects of alternate day feeding and GSH exposure on intact hydras are similar to the effects of GSH and decapitation. Finally, GSH receptors have been demonstrated and characterized in *H. vulgaris* (18).

We propose, therefore, that the tripeptide glutathione plays a central role in the sequence of molecular and cellular events leading to the production of buds in *Hydra*. The sites of action of GSH are, however, not yet evident.

When hydras feed, GSH is presumed to be released from their prey, although this hypothesis has never been validated. Feeding has also been presumed to release a polypeptide with mitogenic and morphogenic activities from endogenous stores in *Hydra* (11). This polypeptide (*i.e.*, 'head activator') is also released by transection of the head or other injury (10) and stimulates both budding and head regeneration. But the rise in the mitotic index subsequent to feeding is more likely to be due to a release from inhibition caused by feeding (19).

Thus, although alternate day feeding and decapitation are equally efficient at stimulating bud initiation, we cannot be sure that their mechanisms of action are identical. As for GSH, it may be a part of the cascade of events initiated by head activator; it may be one of the signals initiating the cascade; or it may act through another pathway altogether.

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